Pergamon

Environmental Toxicology and Chemistry, Vol. 13, No. 11, pp. 1867-1872, 1994
Printed in the USA
0730-7268/94 $6.00 + .00

0730-7268(94)00122-7

COMPARATIVE ACUTE TOXICITIES OF SEVERAL PESTICIDES AND METALS TO *MYSIDOPSIS BAHIA* AND POSTLARVAL *PENAEUS DUORARUM*

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Abstract – Effects of toxic chemicals on estuarine and marine crustaceans are often evaluated using the mysid *Mysidopsis bahia*. In a literature survey of results of acute toxicity tests with estuarine crustaceans, Mysidae and Penaeidae were generally the two most sensitive families. However, neither family was consistently more sensitive (higher LC50 divided by lower LC50 > 2). Mysids were 54 times more sensitive to pyrethroids than were penaeids ($N = 3$ studies). Yet penaeids were 36 times more sensitive ($N = 4$ studies) to organochlorines and 5 times more sensitive ($N = 17$ studies) to organophosphates than were mysids.

Acute exposures of ≤24-h-old mysids and second postlarval pink shrimp *Penaeus duorarum* were conducted to compare responses of these crustaceans to each other, and to values from the literature for other estuarine crustaceans. The test compounds were chloride salts of cadmium, copper, and zinc; the organophosphates diazinon, fenthion, and malathion; and several pyrethroids – cypermethrin, fenvalerate, and permethrin.

Results showed that pyrethroid toxicities to mysids and postlarval pink shrimp were similar, organophosphate toxicities were within a factor of 2.6 for both species, and mysids were 3 to 26 times more sensitive to the metals than were larval pink shrimp.

Keywords – Penaeids Mysids Metals Organophosphates Pyrethroids

INTRODUCTION

In recent years, *Mysidopsis bahia* has often been used as a surrogate for other estuarine crustaceans to screen those toxics having the potential to enter estuarine and marine waters. A review [1] of acute estuarine crustacean exposures compiled in water quality criteria documents [2] and other literature [3–6] found that Penaeidae (*Penaeus aztecus, P. duorarum*, and *P. setiferus*) was most frequently the family with greatest sensitivity to toxic materials. Penaeids were 36 times more sensitive ($N = 4$ studies) to organochlorines and 5 times more sensitive ($N = 17$ studies) to organophosphates than were mysids. However, mysids were 54 times more sensitive ($N = 3$ studies) to pyrethroids than were penaeids. (Evaluations of variability among aquatic toxicity test results indicate that, generally, they are reproducible within a factor of 2 across laboratories [7–9].)

Variability among LC50 test results within a species may be related to the age or molt stage of specimens exposed to the toxicant. Previous studies have shown that shrimp and crab larvae were 1 to 3 orders of magnitude more sensitive to pesticides and metals than were adults [10–12]. Also, cooccurrence of molting and mortality in exposures to toxic materials has been observed previously [10,13–16]. In general, toxicity test shrimp have been large juvenile, subadult, or adult shrimp that would not be as likely as early life stages to molt during an acute exposure.

Postlarval pink shrimp, approximately 4 mm total length (TL), are found in shallow coastal waters such as Florida Bay and Tampa Bay [17–19] where they may be exposed to anthropogenic contaminants. They remain there until late juvenile or early adult stages, then migrate to deeper water and become part of a $492 million (1992) fishery [20]. Comparisons of the sensitivity to contaminants of these postlarvae to those of other estuarine crustaceans and to *M. bahia* could provide a reference for the level of protection afforded this species in contaminant regulation. However, routine testing of very young penaeids indigenous to the United States (*Penaeus aztecus, P. duorarum*, and *P. setiferus*) is restricted by problems associated with field collection and identification, and minimal availability of laboratory-cultured organisms.

The objective of this research was to compare sensitivity of pink shrimp postlarvae (*P. duorarum*) to mysids and other estuarine crustaceans reported in the literature from exposures to three classes of chemicals. Mysids (≤1-d postrelease) and laboratory-cultured 3- to 5-d-old postlarvae of pink shrimp were exposed to each of three organophosphates, three pyrethroids, and three metals under the same conditions.

MATERIALS AND METHODS

The toxicants chosen for the mysid and shrimp comparison testing were the organophosphates diazinon, *O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate; fenthion, *O,O*-dimethyl *O*-[3-methyl-4-(methylthio)phenyl] phosphorothioate; and malathion, *O,O*-dimethyl phosphorodithioate of diethyl mercaptosuccinate. Three pyrethroids were used: cypermethrin, (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate; fenvalerate, (*RS*)-α-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate; and permethrin, 3-(phenoxyphenyl) methyl (±)-*cis,trans*-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate. Chloride salts of cadmium, copper, and zinc were also tested. Pesticides were

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obtained from the U.S. Environmental Protection Agency (EPA) Repository, Research Triangle Park, North Carolina, and the metals from Aldrich Chemical Co. (99.99% pure).

Seawater used for all toxicity tests was pumped from Santa Rosa Sound, Florida, through sand and 1-μm fiber filters, and aerated until it was used. Deionized water was added to dilution water to adjust the salinity to 25‰.

Stock solutions were made by dissolving the pesticides in 90% triethylene glycol and 10% acetone, with solvent controls in the same proportion; the metals were mixed in deionized water. Prior to dosing, pesticides and total metal concentrations were measured using gas chromatography and plasma emission spectrometry.

Mysids were ≤24-h-postrelease juveniles obtained from static laboratory cultures maintained at 25°C and 25‰. Pink shrimp used for testing were laboratory-spawned 3- to 5-d-old postlarvae obtained by techniques described by Cripe (in review) and held at 25 ± 1°C and 25 ± 1‰. Briefly, eggs were collected from eye-enucleated brood stock, fed *Chaetoceros gracilis* for 11 d until molt to postlarvae and then fed 48 h posthydration *Artemia* nauplii for 3 to 5 d prior to exposure.

Static, 96-h acute toxicity tests were conducted with pink shrimp postlarvae and ≤24-h-postrelease mysids using standard techniques for macroinvertebrates [21]. Dissolved oxygen was measured in all treatments at hours 0, 48, and 96. Control salinity and pH in all treatments were measured at the start and end of each test, while test temperature was recorded continuously. Survival was checked, dead animals removed, and survivors fed at 24-h intervals.

The test chambers were 20-cm-diameter glass dishes filled with 990 ml of dilution water. Five toxicant concentrations (60% dilutions) were obtained by adding the appropriate volume of test chemical from a stock solution to the dilution water and were stirred gently to distribute toxicant. All pesticide treatments except the seawater control received the same carrier solvent concentration (100 μl/L). Both carrier solvent and seawater controls were used in all pesticide tests, and only a seawater control was used in the metal tests.

For each test, two replicates of 10 animals randomly distributed were exposed to each treatment. The animals were transferred to the assigned exposure vessel and fed 48-h posthydration *Artemia* nauplii at a density of approximately 60 per test organism. The replicate test chambers of each concentration were covered and placed in an incubator without aeration at 25 ± 1°C, in a 14:10-h light:dark photoperiod.

All LC50 estimates were calculated by the trimmed Spearman–Karber statistical method [22], with Abbott's correction for control mortality, when needed.

RESULTS

Water-quality parameters in all tests remained within limits set by the American Society for Testing and Materials [21]. Salinity was 25‰ and temperature was 25 ± 0.5°C for all tests. Mean dissolved oxygen concentration at test termination for mysid exposures was 5.9 (SD = 0.34) mg/L with a minimum of 4.3 mg/L, and for postlarval shrimp exposures was 5.6 (SD = 0.64) mg/L with a minimum of 4.3 mg/L. By test termination, pH ranged from 7.8 to 8.1 for all tests except exposures of *P. duorarum* to diazinon (7.5–7.9), cypermethrin (7.6–7.9), and permethrin (7.5–7.9).

Results of exposures of *M. bahia* and *P. duorarum* to nine chemicals are listed in Table 1 and contrasted with historical data. Seawater and solvent-control results were pooled for calculation of the LC50 in pesticide exposures of both mysids and shrimp. Mysid control mortality averaged 3% (SD = 3.6), and in only one test exceeded 5% (in the cadmium chloride exposure, 12.5% of control animals died). Mean shrimp-control mortality was 4% (SD = 4.7), and in only one test exceeded 10%. (In the permethrin exposure, 15% of shrimp in the solvent control died, yet none died in the seawater-control treatment and only 5% died in the lowest concentration.)

Mysids and penaeids differed in time-to-death in the 96-h tests. Virtually all mysid mortality occurred within 48 h in a concentration-dependent relationship, with the exception of zinc where mortality continued until 72 h. The LC50s calculated from 48 and 72 h differed from the 96-h LC50 by an average of 17%. Pink shrimp mortality was generally concentration dependent, but did not subside until 72 h of exposure, except for the cypermethrin exposure where mortalities occurred within 24 h and in fenthion and permethrin exposures where it occurred within 48 h. The LC50s for pink shrimp calculated for 48 and 72 h differed from the 96-h LC50 by an average of 34%. With both species, mortality frequently coincided with molting, identified by appearance of discarded exoskeletons in the exposure vessel.

Mysid and pink shrimp LC50s calculated from static acute exposures to pesticides were similar. Fenthion and fenvalerate were slightly (two to three times) more toxic to shrimp, and diazinon and cypermethrin had more effect on mysids. In all exposures to metals, however, mysids were consistently more sensitive.

DISCUSSION

For the life stages tested, mysids and pink shrimp are epibenthic in habit and are commonly found in the estuary at 15 to 28‰ and 23 to 29°C [17–19,23,24]. Test animals were similar in size (mysids, approx. 3 mm TL and pink shrimp, approx. 7 mm TL) and, with the exception of metal exposures, responded similarly to most of the contaminants used in this study.

The time of death for both test species corresponded to the pattern of molting. Mysids (≤24 h postrelease) molt at 48 h at 25°C, and most mysid deaths occurred at 48 h. Pink shrimp postlarvae (3 d after metamorphosis) molt at 48 or 72 h at 25°C, and shrimp mortality did not stabilize until 72 h of contaminant exposure. Death at molting has been seen previously in *Macrobrachium rosenbergii* exposed to nitrite [13], exposures of *Penaeus kerathurus* (Forskal) to chlorine [16], and juvenile and adult pink shrimp exposed to Aroclor® 1254 [10,14]. The data reported here also suggest an increase in sensitivity of crustaceans to toxicants during molting.

More frequent molting seen in younger stages of aquatic crustaceans may explain some of the sensitivity differences between adults and larvae of the same species. Larval crabs (*Cancer magister* and *Carcinus maenas*) as well as the sand

Table 1. Acute 96-h LC50s for estuarine crustaceans exposed to three organophosphates, three pyrethroids, and three metal chloride salts

Chemical and species tested	Life stage	Exposure method	LC50 (C.I.) (μg/L)	Reference no.
Diazinon				
Mysidopsis bahia	J[a]	S[b]	8.5 (8.2–8.9)	This study
M. bahia	J	FT[b]	4.82 (4.21–4.92)	36
Penaeus duorarum	PL[a]	S	21 (19–24)	This study
Fenthion				
P. duorarum	J	FT	0.11 (0.09–0.12)	37
M. bahia	J	FT	0.15 (0.12–0.19)	37
P. duoarum	PL	S	0.21 (0.17–0.25)	This study
M. bahia	J	S	0.56 (0.48–0.65)	This study
Malathion				
M. bahia	J	FT	2.6 (2.3–2.9)	37
M. bahia	J	S	5.4 (5.1–6.4)	38
Palaemonetes pugio	A[a]	SR[b]	10.4 (8.4–12.9)	39
M. bahia	J	S	11 (9.1–11)	This study
P. duorarum	PL	S	12 (10–13)	This study
Crangon septemspinosa	J	S	33 (No C.I.)	40
Palaemonetes vulgaris	A	S	82 (No C.I.)	40
Pagurus longicarpus	J	S	83 (No C.I.)	40
Cypermethrin				
C. septemspinosa	J	SR	0.01 (No C.I.)	41
P. pugio	A	FT	0.016 (No C.I.)	42
M. bahia	J	S	0.018 (0.014–0.023)	38
M. bahia	J	S	0.027 (0.024–0.031)	This study
Homarus americanus	J	SR	0.04 (No C.I.)	41
P. duorarum	PL	S	0.11 (0.089–0.13)	This study
Fenvalerate				
M. bahia	A	FT	0.008 (0.005–0.01)	43
P. duorarum	PL	S	0.012 (0.010–0.015)	This study
P. pugio	A	SR	0.013 (0.009–0.018)	39
M. bahia	J	S	0.032 (0.028–0.038)	This study
C. septemspinosa	J	SR	0.04 (No C.I.)	41
P. pugio	A	SR	0.052 (0.043–0.063)	44
P. pugio	PL	SR	0.071 (0.049–0.102)	29
H. americanus	J	SR	0.14 (No. C.I.)	41
P. duorarum	A	FT	0.84 (0.66–1.2)	43
Permethrin				
M. bahia	A	FT	0.02 (0.017–0.024)	20
M. bahia	J	S	0.095 (0.077–0.12)	This study
C. septemspinosa	J	SR	0.13 (No C.I.)	41
P. duorarum	PL	S	0.17 (0.15–0.19)	This study
P. duorarum	A	FT	0.22 (0.06–0.79)	20
H. americanus	J	SR	0.73 (No C.I.)	41
Cadmium chloride				
M. bahia	J	S	19.6 (17.3–22.1)	This study
P. duorarum	PL	S	509 (407–635)	This study
Copper chloride				
M. bahia	J	S	153 (134–175)	This study
P. duorarum	PL	S	832 (726–955)	This study
Zinc chloride				
M. bahia	J	S	303 (253–362)	This study
P. duorarum	PL	S	1,050 (938–1,170)	This study

Data from the literature include all significant figures reported.
[a]J = juvenile; PL = postlarvae; A = adult.
[b]FT = flow-through test; S = static; SR = static renewal.

shrimp (*Crangon crangon*) were 1 to 2 orders of magnitude more sensitive than adults to metals or methoxychlor [11,12]. Results of the current study show that 3-d-old postlarval pink shrimp are much more sensitive to total cadmium or fenvalerate than are juveniles or adults tested previously in flow-through exposures (LC50 = 3,500 μg Cd/L, [25] and Table 1), which generally produce lower LC50s than do static exposures.

Direct comparison of chemical sensitivity between mysids and pink shrimp postlarvae was facilitated by the use of static exposures even though some mysid data existed previously from flow-through testing. Comparisons of data from this study to existing crustacean data should be made carefully since flow-through exposures provide continuous infusion of contaminated dilution water, eliminating problems of test-chemical depletion by sorption to exposure vessels and food, and uptake by test animals experienced in static tests. However, within an exposure technique, mysids were most frequently as sensitive or more sensitive than other estuarine crustaceans tested with these chemicals.

In results obtained in this study, mysids and shrimp responded similarly to organophosphates, differing from each other by 2.6 times or less. In exposures to malathion, both species were substantially more sensitive than were some other estuarine crustaceans (Table 1).

Laboratory data can be compared to field-effect concentrations of fenthion and malathion from previous mosquito-control studies. Tagatz et al. [26] and Borthwick et al. [27] found no effect on blue crabs, grass shrimp, or pink shrimp exposed to malathion, decreasing from 1.1 to 0.1 μg/L (concentrations well below the LC50 for the grass and pink shrimp, Table 1). In mosquito-control applications, Clark et al. [28] saw no mysid or pink shrimp mortality in fenthion-dosed waters, decreasing from 1.5 μg/L to 0.12 μg/L in 2 h, but saw significant death in initial concentrations of 2.6 μg fenthion/L that decreased to 1.4 μg/L in 6 h. Both of these initial concentrations are clearly in excess of laboratory-static 96-h LC50s for mysids and pink shrimp postlarvae or juveniles (0.11–0.56 μg fenthion/L, Table 1). These extremely short exposures to high concentrations of fenthion evidently are tolerated. In a laboratory simulation of field pulsed exposures, total mortality of mysids occurred at high initial concentrations of 2.6 μg fenthion/L, but none occurred in $\leq$1.0 μg fenthion/L [4]. These pulse studies provide some insight into tolerance of estuarine crustaceans to varying concentration of poisons and the application of static toxicity data to field exposures.

In contrast to the similarity seen in responses of mysids and shrimp to organophosphates, pyrethroid exposure was not as predictable. In static exposures, pink shrimp postlarvae and mysids treated with fenvalerate or permethrin differed from each other again by 2.6 times or less, but mysids were 4 times more sensitive to cypermethrin than were pink shrimp postlarvae. Among the pyrethroids, mysids were either the most sensitive species tested or differed from the most sensitive by 2.6 times or less.

Comparison of laboratory results to field concentrations of pyrethroids suggests a potential for impact on survival of estuarine crustaceans in the absence of pulsed-dose information. After rainfalls, Baughman et al. [29] measured agricultural field runoff into estuaries and found 0.106 μg fenvalerate/L in 23‰ salinity and 0.079 μg fenvalerate/L in 26‰ salinity. (Static LC50s for mysids and postlarval shrimp range from 0.013 to 0.032 μg/L.) Carroll et al. [30] detected permethrin in agricultural runoff at $\leq$ 0.2 μg/L. (Static LC50s for mysids and postlarval shrimp range from 0.095 to 0.17 μg/L.) Additional testing of estuarine crustaceans is needed to document ranges of short-term tolerances of non-target species to these chemicals.

A thorough review of exposures of a variety of estuarine crustaceans to cadmium, copper, and zinc can be found in water quality criteria documents [31–33]. Because of the volume of the data, the results were not included in Table 1 but will be discussed generally for comparison to data from this study. The mysid LC50 of 19.6 μg cadmium chloride/L reported here is similar to that found by others (17 to 32.8 μg cadmium chloride/L [31,34,35]). Toxicity of zinc chloride to mysids in this study (303 μg/L) is also similar to those reported previously (ranging from 499 to 547 μg/L [33]).

Responses of pink shrimp postlarvae and mysids to metals exposure are consistently different. Pink shrimp postlarvae are 3 to 25 times less sensitive than mysids to the metals tested here. Mysids are also 1 to 3 orders of magnitude more sensitive to cadmium than are grass shrimp, amphipods, or crabs [31] and 1 to 3 orders of magnitude more sensitive to zinc than the amphipod *Corophium volutator* and the crab *Carcinus maenas* [33]. Copper, on the other hand, is 3 times more toxic to *Homarus americanus* and *Cancer magister* [32] than to *M. bahia*, which is 5 times more sensitive than pink shrimp postlarvae.

In conclusion, mysids were similar to pink shrimp postlarvae in sensitivity to most test pesticides, which contrasts to earlier results with juvenile or adult pink shrimp. Also, there is some additional indication of greater sensitivity of younger stages of shrimp to some chemicals than older test organisms. The data compared in this study suggest that mysids are good indicators of minimum lethal concentrations of chemicals for many estuarine crustaceans, including commercially important species, and support their use in regulatory programs designed to control these toxic substances.

Acknowledgement – I gratefully acknowledge Patricia Gordon and Mau T. Nguyen for assistance with some acute exposures. Laboratory staff member C.R. Cripe provided invaluable editorial assistance and J. Forester and E.M. Lores provided chemical analyses.

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